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Restriction Maps of Five Autographa Californica MNPV Variants, Trichoplusia Ni MNPV, and Galleria Mellonella MNPV DNAs with Endonucleases SmaI, KpnI, BamHI, SacI, XhoI, and EcoRI

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16. ABSTRACT

The restriction sites of Autographa californica nuclear polyhedrosis virus (AcMNPV) L DNA were mapped for the endonucleases SmaI, KpnK, BamHI, SacI, XhoI, and EcoRI. The restriction maps of four other AcMNPV variants, Trichoplusia ni (TnMNPV), and Galleria mellonella (GmMNPV) genomes were determined and compared to the endonuclease cleavage maps of AcMNPV E_2 DNA. The viral structural polypeptides of AcMNPV variants S_3, E_2, S_1, M_3, and R9 were the same when analyzed by polyacrylamide gel electrophoresis. The major structural polypeptides of GmMNPV and TnMNPV had the same pattern in polyacrylamide gels as did AcMNPV structural polypeptides. GmMNPV and TnMnPV had several minor structural protein differences as compared with AcMNPV, AcMNPV variants, TnMNPV, and GmMNPV were distinct but with very similar genomes and protein structures.

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Restriction Maps of Five *Autographa californica M*NPV Variants, *Trichoplusia ni M*NPV, and *Galleria mellonella M*NPV DNAs with Endonucleases *Sma*I, *Kpn*I, *Bam*HI, *Sac*I, *Xho*I, and *Eco*RI

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The restriction sites of *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV) E2 DNA were mapped for the endonucleases *Sma*I, *Kpn*I, *Bam*HI, *Sac*I, *Xho*I, and *Eco*RI. The restriction maps of four other Ac*M*NPV variants, *Trichoplusia ni* (Tn*M*NPV), and *Galleria mellonella* (Gm*M*NPV) genomes were determined and compared to the endonuclease cleavage maps of Ac*M*NPV E2 DNA. The viral structural polypeptides of Ac*M*NPV variants S3, E2, S1, M3, and R9 were the same when analyzed by polyacrylamide gel electrophoresis. The major structural polypeptides of Gm*M*NPV and Tn*M*NPV had the same pattern in polyacrylamide gels as did Ac*M*NPV structural polypeptides. Gm*M*NPV and Tn*M*NPV had several minor structural protein differences as compared with Ac*M*NPV. Ac*M*NPV variants, Tn*M*NPV, and Gm*M*NPV were distinct but with very similar genomes and protein structures.

The nuclear polyhedrosis virus of *Autographa californica* (Ac*M*NPV) is an insect baculovirus with a circular DNA genome of about 80×10^6 daltons. Site-specific endonucleases have recently been used to study the structure and function of baculovirus genomes, in particular, that of Ac*M*NPV (3–5, 8). In general, restriction endonucleases have led to advances in virus identification, the mapping of genes, DNA sequence analysis, novel recombinant DNA research, study of viral recombination, and gene isolation. Ac*M*NPV is of interest in that it has a relatively broad in vivo and in vitro host range and is the baculovirus that had been the most extensively characterized in terms of its structure and biological properties in insect cell culture (8, 11, 13). In addition, baculovirus genomes have a yet unexplored potential as cloning vectors [Fed. Regist. 43:33042–33178] and are being considered as natural agents for the control of insect pests.

Two independent reports have shown that wild isolates of Ac*M*NPV derived from infected insects can be separated by plaque purification into genomic variants (3, 8), which have slightly different restriction enzyme fragment patterns. The physical mapping and ordering of endonuclease cleavage sites would enable interpretation of the differences in fragment patterns of Ac*M*NPV variants. In this study, we describe the cleavage patterns of five variants of Ac*M*NPV, as well as the nuclear polyhedrosis viruses from *Galleria mellonella* (Gm*M*NPV) and *Trichoplusia ni* (Tn*M*NPV), produced by restriction enzymes *Sma*I, *Kpn*I, *Bam*HI, *Sac*I, *Xho*I, and *Eco*RI. The cleavage sites have been mapped, and the restriction fragments have been ordered, resulting in a more precise understanding of the genome structure and relationships among these seven closely related baculovirus DNAs.

MATERIALS AND METHODS

Cells and virus. A continuous cell line of *T. ni*, TN-368, was used for preparation of viral isolates. The cell line was maintained as described in a previous study (12).

Variants S1, E2, M3, and S3 of Ac*M*NPV were isolated as described previously (8). The Ac*M*NPV variant R9 was plaque purified from a *Rachiplusia ou* nuclear polyhedrosis virus wild isolate obtained from Clint Kawanishi (Environmental Protection Agency, Research Triangle Park, N.C.). Non-plaque-purified isolates of Tn*M*NPV and Gm*M*NPV were kindly provided by Art McIntosh (Rutgers University, New Brunswick, N.J.). Purification of virus and isolation of polyhedra for analysis of structural polypeptides were done as described previously (10). To analyze the viral structural polypeptides, purified virus was disrupted in 1.0% sodium dodecyl sulfate and 5.0% 2-mercaptoethanol at 100°C for 3 min. Samples containing 30 to 40 μg of viral protein were electrophoresed in 11% polyacrylamide gel slabs in the presence of 0.1% sodium dodecyl sulfate as described by Laemmli (2) as modified for baculovirus preparations (10).

Preparation and purification of [^{32}P]DNA. Viral DNAs were labeled with radioactive isotopes in

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infected TN-368 cells by a modification of the method reported by Volkman et al. (13). A cell monolayer was established by seeding 150-cm^2 Corning tissue culture flasks with 2×10^7 TN-368 cells and allowing attachment to occur for 1 h in medium minus fetal calf serum. The cells were inoculated with extracellular virus at a multiplicity of infection of 5 to 10 PFU per cell in 5 ml of phosphate-free medium (8). After gently rocking the infected monolayers for 1 h, the inoculum was removed and replaced by phosphate-free medium containing 50 μCi of carrier-free $^{32}P_i$ (50 Ci/mmol, New England Nuclear Corp., Boston, Mass.) per ml. At 72 h postinfection, viral [^{32}P]DNA was purified essentially as described previously (8). The infected cells were removed from the labeling medium by centrifugation at 2,000 $\times$ *g* for 15 min suspended in 0.01 M Tris–0.001 M EDTA, pH 7.5. To disrupt cell membranes and release polyhedra, Pronase was added to a final concentration of 1.0 mg/ml, and the preparation was incubated for 15 min at room temperature. The polyhedra banded at a density of 1.25 g/ml in 40 to 63% (wt/wt) sucrose gradients after centrifugation for 30 min at 100,000 $\times$ *g*. The polyhedral crystals were dissolved at a concentration of 5 mg of polyhedra per ml in 0.1 M $NaCO_3$–0.17 M NaCl–0.01 M EDTA, pH 10.9, by incubation at 37°C for 15 min. Sodium dodecyl sulfate and proteinase K were added to 1% and 0.5 mg/ml, respectively, and the solution was incubated for an additional 60 min at 37°C. The DNA was extracted three times with redistilled phenol saturated with 0.01 M Tris–0.001 M EDTA, pH 7.5, and once with chloroform–isoamyl alcohol (24:1). This was dialyzed extensively against 0.1 $\times$ SSC (1$\times$ SSC = 0.015 M sodium citrate plus 0.15 M NaCl, pH 7.5).

Restriction endonucleases. *Bam*HI, *Eco*RI, *Sac*I, *Sma*I, and *Xho*I restriction endonucleases were purchased from New England Biolabs (Beverly, Mass.). All restriction enzyme digests were done in 10 mM Tris–50 mM NaCl–10 mM $MgCl_2$–10 mM 2-mercaptoethanol, pH 7.5, except for *Sma*I, which required 15 mM Tris–15 mM KCl–6 mM $MgCl_2$, pH 8.5. Enzyme incubation was carried out at 37°C for 2 to 3 h with demonstrated excess enzyme to achieve a limit digestion. Lambda DNA (0.5 μg) was added to all [^{32}P]DNA digests as an internal standard to confirm that limit digestion had occurred.

Double digests were done by digesting virus DNA with one of the restriction enzymes; this mixture was extracted with buffer-saturated phenol and subsequently dialyzed in 0.1$\times$ SSC. The viral DNA fragments were then digested with the second restriction enzyme.

Agarose gel electrophoresis and autoradiography. Each restriction enzyme reaction mixture was stopped by adding 0.1 volume of a solution containing 0.1 M EDTA, 50% glycerol, and 0.5% bromophenol blue. The samples were electrophoresed in a 0.75% agarose horizontal slab gel for 24 h at 50 V as described previously (8). For autoradiography, the gel slabs were dried under vacuum, and the ^{32}P-labeled fragments were detected by exposure to Kodak NS-54T X-ray film with a Dupont Lightning-Plus intensifying screen at −70°C for 1 to 2 days.

Purification of DNA fragments. Individual restriction fragments were isolated from agarose gels after electrophoresis of 0.5 to 1.0 μg of digested [^{32}P]DNA as described for analytical gels. Often, electrophoresis was continued for 48 to 72 h to increase separation of high-molecular-weight fragments. The UV-visible bands were cut from the gel, and the DNA was simultaneously removed and concentrated by electrophoresis to approximately 0.2 ml as described by Allington et al. (1). The electrophoretic extraction and concentration was done for 3 h at 100 V in 0.01 M Tris buffer containing 0.05 M glycine and 0.1% sodium dodecyl sulfate, pH 8.6. The recovery of ^{32}P as measured directly by the Cerenkov procedure was greater than 90%. Sonicated calf thymus DNA was added to a final concentration of 50 μg/ml, and each DNA fragment was phenol extracted. Extracted fragments were adjusted to a final concentration of 0.15 M NaCl and precipitated by addition of two volumes of 100% ethanol and storage at −20°C overnight. DNA precipitates were pelleted by centrifugation at 10,000 $\times$ *g* for 30 min. The pellets were washed with 70% ethanol, and excess alcohol was removed by lyophilization. The DNA fragments recovered by this procedure were suspended in 0.1$\times$ SSC.

In vitro labeling of lambda DNA molecular weight standards. The molecular weights of baculovirus restriction fragments were determined by comparison with the mobilities of ^{32}P-labeled lambda DNA restriction fragments (8). Lambda DNA was restricted, and the fragments were labeled intact by a modification of the nicktranslation procedure of Rigby et al. (7). The reaction mixture (100 μl) was: 0.05 M Tris; 0.005 M $MgCl_2$; 0.01 M 2-mercaptoethanol, pH 7.8; 5 μg of bovine serum albumin; 0.001 mM each dCTP, dGTP, dTTP, and [α-^{32}P]ATP (specific activity, 300 to 400 Ci/mmol); 1.0 μg of lambda DNA *Eco*RI, *Hin*dIII, or *Sma*I restriction fragments; and 2 U of DNA polymerase I (New England Biolabs). Incubation was carried out at 14°C for 4 to 6 h. The reaction was stopped by adding EDTA to 0.01 M, and the mixture was immediately extracted with phenol. Unincorporated [α-^{32}P]ATP was removed by twice precipitating the labeled DNA with 70% ethanol. Restriction fragments labeled in this manner had specific activities of 1×10^7 to 2×10^7 cpm/μg and had the same mobilities as unlabeled lambda restriction fragments.

RESULTS

Method for constructing restriction maps. The procedure used to establish the order of Ac*M*NPV DNA restriction fragments involved the reciprocal double digestion of isolated DNA fragments (6). Reciprocal digestion with two restriction enzymes should yield the same set of end fragments, defined as fragments each having one end produced by the first enzyme and the other produced by the second restriction enzyme. A sequential order of fragments from two restriction enzymes which overlap each other can be determined by connecting each band cleavable by the other enzyme to its set of end fragments. Reciprocal digestion experiments were conducted with both Ac*M*NPV E2 and

Ac*M*NPV S1 variants, and the order of *Eco*RI, *Bam*HI, *Sma*I, *Xho*I, *Kpn*I, and *Sac*I restriction fragments was determined. A complete set of data necessary to map the restriction fragments of one of these variants, E2, is presented in Table 1 and Fig. 1 through 7. The restriction maps of the DNAs from M3, S3, and R9 variants of Ac*M*NPV as well as Tn*M*NPV and Gm*M*NPV were interpreted by direct comparisons of the limit digests with single restriction enzymes to those of Ac*M*NPV E2. Any ambiguities in the restriction maps of these closely related DNAs were confirmed by selective double digestion experiments.

Single and double restriction endonuclease digests of Ac*M*NPV E2. The autoradiograms of Ac*M*NPV E2 [^{32}P]DNA fragments after digestion with either *Kpn*I, *Bam*HI, *Xho*I, *Eco*Ri, *Sma*I, or *Sac*I restriction endonuclease and electrophoresis in agarose gels are shown in Figure 1. Each band was lettered sequentially by increasing mobility. Fragments which comigrated were given double-letter designations, e.g., *Eco*RI fragments BC and UV. The molecular weight of each restriction fragment of Ac*M*NPV E2 DNA (Table 1) was determined as described above. Ac*M*NPV E2 DNA had 4 *Sma*I, 5 *Kpn*I, 7 *Bam*HI, 9 *Sac*I, 13 *Xho*I, and 23 *Eco*RI restriction enzyme cleavage sites. The sums of the molecular weights of Ac*M*NPV E2 restriction fragments were each approximately 81.9×10^6 daltons. DNA fragments of less than 0.3 megadaltons, or about 0.4% of the genome, would have electrophoresed off the gel.

To order fragments, first, double digests of Ac*M*NPV E2 [^{32}P]DNA were done as described in Materials and Methods and electrophoresed in agarose gels. Double digests simplified the

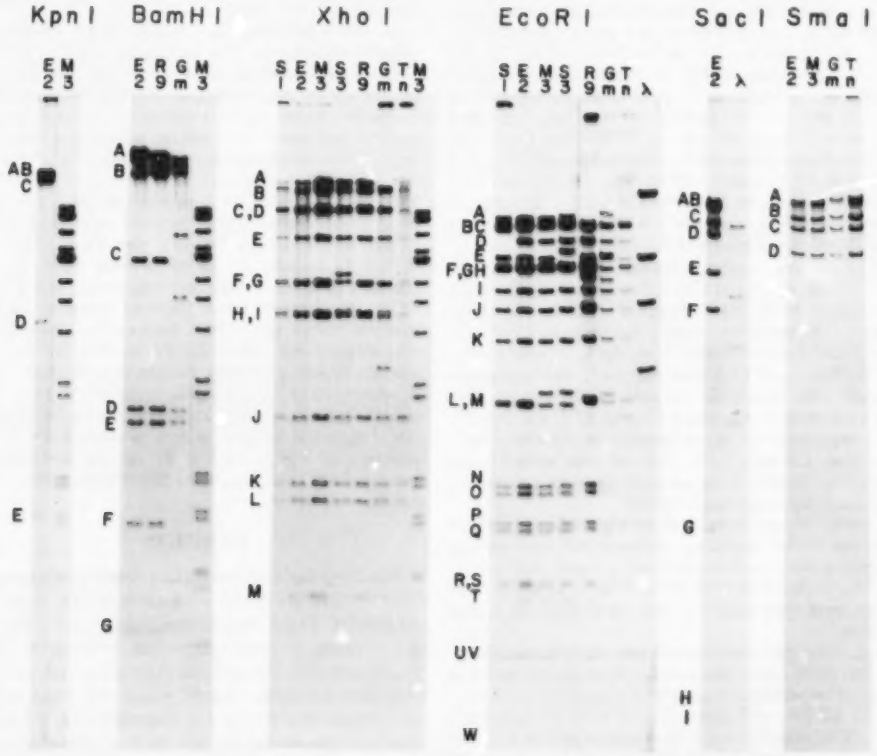

FIG. 1. *Autoradiograms of electrophoretically separated ^{32}P-labeled fragments of AcMNPV variants S3, E2, S1, M3 and R9, GmMNPV (Gm), and TnMNPV (Tn). Each of the seven virus DNAs was restricted with KpnI, BamHI, XhoI, EcoRI, SacI, or SmaI and electrophoresed in 0.75% agarose. In the KpnI, BamHI, SacI, and SmaI digests of these seven virus DNAs, the restriction patterns of those viruses not shown were the same as those represented by AcMNPV E2. An EcoRI digest of AcMNPV M3 is in the rightmost slots of KpnI, BamHI, and XhoI digest for comparison. HindIII fragments of lambda [^{32}P]DNA (λ) were electrophoresed in slots adjacent to the EcoRI and SacI digests. Restriction bands of AcMNPV E2 have been lettered as described in the text.*

TABLE 1. *Molecular weights of AcMNPV E2 restriction fragments*

Fragment	Mol wt[a] (megadaltons)					
	*Eco*RI	*Xho*I	*Sac*I	*Bam*HI	*Kpn*I	*Sma*I
A	9.0	18.8	20.0	55.3	32.4	45.4
B	8.2	14.7	20.0	15.0	26.5	16.1
C	8.0	9.3	14.5	5.4	18.1	12.2
D	7.0	9.1	10.8	2.25	3.6	8.2
E	6.0	6.8	7.5	2.10	1.3	
F	5.7	4.9	5.6	1.20		
G	5.6	4.8	1.8	0.60		
H	5.6	3.9	0.75			
I	4.8	3.8	0.68			
J	4.1	2.15				
K	3.4	1.52				
L	2.5	1.40				
M	2.45	0.74				
N	1.55					
O	1.50					
P	1.30					
Q	1.20					
R	0.88					
S	0.86					
T	0.78					
U	0.53					
V	0.53					
W	0.38					

[a] Molecular weights of restriction fragments were each the mean of three independent determinations. Molecular weights of fragments greater than 10.0 megadaltons were calculated from the sizes of restriction fragments after digestion of isolated fragments with *Eco*RI.

mapping procedure in that (i) the end fragments used to link overlapping restriction fragments of two restriction enzymes could be determined and (ii) the restriction fragments of the first enzyme cleaved by the second enzyme were found, thus eliminating the need to purify and digest those restriction bands not cleaved by the second restriction endonuclease.

A representative number of reciprocal double digests has been selected (Fig. 2 through 7) that provide adequate information to order AC*M*NPV E2 fragments produced by the six restriction endonucleases. To simplify the reciprocal digest figures (Fig. 2 through 7), only those DNA bands that are limit digest fragments have been labeled with respect to their weight in megadaltons. Unlabeled fragments are those not cleaved by the second restriction enzyme, partial digest products, or contaminants of adjacent restriction fragments (none of these interfered with the analysis of the data).

A detailed description of the reciprocal double digest data for *Bam*HI and *Eco*RI and the order of *Bam*HI fragments of Ac*M*NPV E2 DNA is given below to illustrate how the order of all restriction fragments was determined.

Mapping of *Bam*HI cleavage sites. Cleavage of Ac*M*NPV E2 DNA with BamHI gave seven fragments, *Bam*HI-A through *Bam*HI-G (Fig. 1 and Table 1). *Bam*HI-A was 68% of the genome in size and was the largest single fragment produced by the six restriction enzymes used in this study. Thus, it was decided to use the *Bam*HI-A cleavage site as the origin (0.0% map location) for aligning the restriction fragments. Electrophoretic separation of E2 DNA restricted with both *Bam*HI and *Eco*RI (Fig. 2)

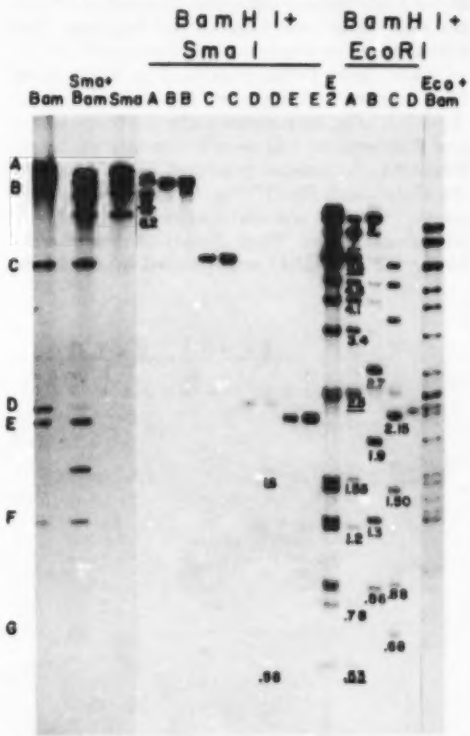

FIG. 2. *Electrophoretically separated fragments of AcMNPV E2 restricted with BamHI (Bam), BamHI + SmaI (Sma + Bam), SmaI (Sma), and EcoRI + BamHI (Eco + Bam). Isolated BamHI fragments A through E were restricted with SmaI (BamHI + SmaI). BamHI fragments B, C, D, and E are shown undigested and digested with SmaI (left and right, respectively, of each pair). Isolated BamHI fragments A through D were restricted with EcoRI (BamHI + EcoRI), and an EcoRI digest of AcMNPV E2 (E2) is electrophoresed in an adjacent slot. Each restriction fragment which was identified as a limit digest produced in reciprocal digest experiments has been labeled as to its molecular weight × 10^{-6}, all other fragments have been left unlabeled. An underlined molecular weight indicates two fragments which comigrated.*

resulted in 30 restriction fragments of which 8 could be identified as end fragments. This same set of end fragments was found when isolated *Bam*HI fragments were restricted with *Eco*RI (Fig. 2) or when isolated *Eco*RI fragments were restricted with *Bam*HI (Fig. 3). The fragments produced by *Eco*RI cleavage of *Bam*HI-A were *Eco*RI fragment BC, D, F, GH, J, K, L, M, N, Q, T, and UV and end fragments of 7.0 and 4.8 megadaltons (Fig. 2). The end fragments were identified in that two restriction bands migrated at 7.0 megadaltons; thus, one was *Eco*RI-D and the other must have been an end fragment. The 4.8-megadalton fragment could not be *Eco*RI-I, as it was cleaved with *Bam*HI; thus, it also must have been an end fragment. *Bam*HI cleavage of *Eco*RI-A (Fig. 3) produced the 7.0-megadalton end fragment as well as a 2.15-megadalton end fragment. Fragments produced by cleavage of *Bam*HI-C with *Eco*RI (Fig. 2) were *Eco*RI fragments O and R and end fragments of 2.15 and 0.68 megadaltons. Thus, *Bam*HI-A was continuous with *Bam*HI-C and spanned by *Eco*RI-A. The 0.68-megadalton end fragment of *Bam*HI-C cleaved with *Eco*RI was also found in the cleavage of *Eco*RI-I with *Bam*HI (Fig. 3), which in addition produced a 2.7-megadalton end fragment plus a *Bam*HI-F fragment of 1.2 megadaltons. The *Eco*RI cleavage products of *Bam*HI-B (Fig. 2) were *Eco*RI fragments BC, P, and S and end fragments of 2.7 and 1.9 megadaltons. Thus, *Bam*HI-F-B was continuous with *Bam*HI-A-C. *Bam*HI cleavage products of *Eco*RI-GH (Fig. 3) were *Bam*HI fragments D and G and end fragments of 1.9 and 0.68 megadaltons. *Bam*HI-E, when cleaved with *Eco*RI, also had a 0.68- as well as a 1.35-megadalton end fragment (not shown). *Bam*HI cleaved *Eco*RI-E into the 1.35-megadalton end fragment and a 4.8-megadalton fragment (Fig. 3) which had been shown to be an *Eco*RI end fragment of *Bam*HI-A. These data established the order of *Bam*HI fragments as A-C-F-B-(D, G)-E. The order of *Bam*HI fragments D and G was determined by reciprocal double digests with *Sma*I. *Bam*HI-D was cleaved by *Sma*I (Fig. 2), and

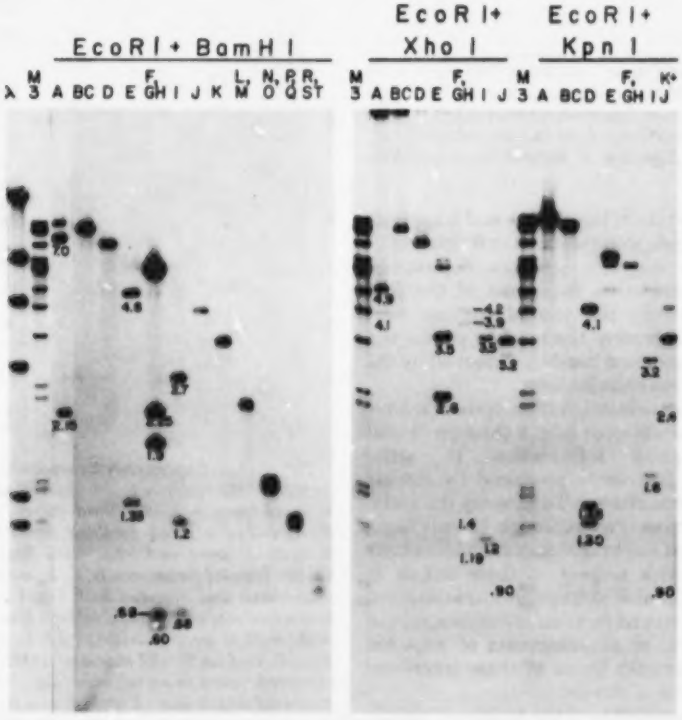

FIG. 3. *Autoradiograms of purified AcMNPV E2 EcoRI restriction fragments that were digested with BamHI (EcoRI + BamHI), XhoI (EcoRI + XhoI), or KpnI (EcoRI + KpnI) and electrophoresed in 0.75% agarose. EcoRI digests of AcMNPV M3 (M3) and the HindIII digest of lambda DNA (λ) were electrophoresed in the indicated slots as standards. Fragments are labeled as in Fig. 2.*

*Bam*HI-G was within *Sma*I-B. Once the order of *Sma*I fragments was established, the order of *Bam*HI fragments D and G could be deduced (see Fig. 8A) as A-C-F-B-D-G-E. Digests of isolated *Bam*HI fragments with *Xho*I, *Kpn*I, *Sac*I, and *Sma*I and reciprocal digests with *Bam*HI resulted in data (not shown) consistent with this order of *Bam*HI fragments.

SmaI cleavage sites. Ac*M*NPV E2 DNA when cleaved by *Sma*I gave four fragments, *Sma*I-A through *Sma*I-D, whose order was determined by reciprocal digests with *Eco*RI. The four *Sma*I cleavage sites were located in *Eco*RI fragments BC, D, F, and GH, yielding eight end fragments (Fig. 4). These same end fragments could be found in the cleavage of *Sma*I fragments A, B, C, and D with *Eco*RI (Fig. 5). An analysis of these data, similar to that described for *Bam*HI above, established the order of *Sma*I restriction fragments as B-C-D-A. This order was confirmed by reciprocal digests with *Bam*HI, *Xho*I, and *Sac*I. Digestion of *Bam*HI-A

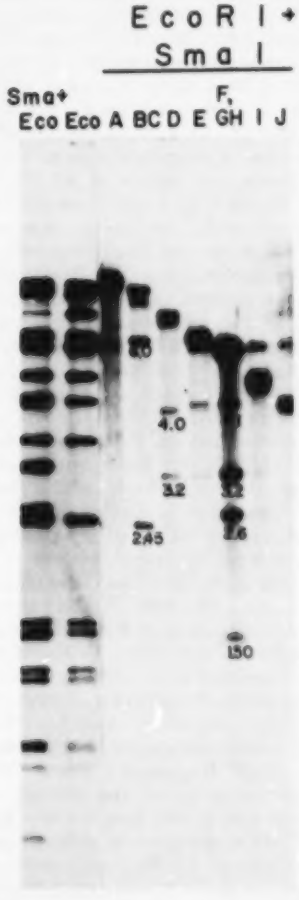

FIG. 4. *AcMNPV E2 DNA was digested with SmaI and EcoRI (Sma + Eco), EcoRI (Eco), and isolated EcoRI fragments A through J were digested with SmaI (EcoRI + SmaI) and then electrophoresed in 0.75% agarose. Fragments are labeled as in Fig. 2.*

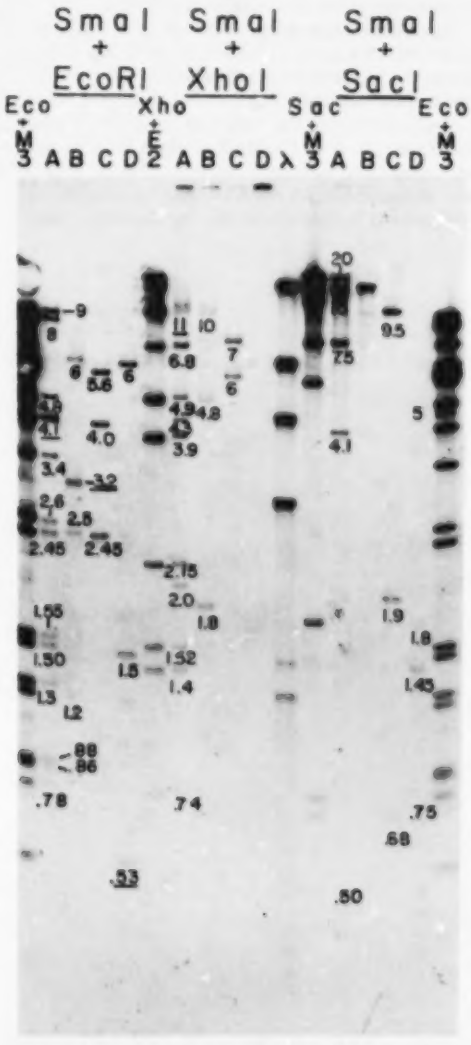

FIG. 5. *Autoradiogram of purified SmaI A through D fragments of AcMNPV E2 digested with EcoRI (SmaI + EcoRI), XhoI (SmaI + XhoI), or SacI (SmaI + SacI) and then electrophoresed in 0.75% agarose. Molecular weight standards electrophoresed in adjacent slots are labeled as described in Fig. 1. Fragments are labeled as in Fig. 2.*

with *Sma*I (Fig. 2) yielded *Sma*I fragments C and D and two end fragments, one of 12 megadaltons (or 14.1% of the genome). This established the position of the *Sma*I-C restriction site as being at 14.1% relative to the *Bam*HI-A cleavage site of 0.0% (see Fig. 8A). The orientations of *Sac*I, *Xho*I, *Eco*RI, and *Kpn*I restriction sites relative to the *Bam*HI-A restriction site (0.0%) were determined in a similar manner.

SacI cleavage sites. The order of *Sac*I restriction sites on Ac*M*NPV E2 DNA was determined by reciprocal digests with *Sac*I and *Eco*RI restriction enzymes (Fig. 6). The data from these experiments gave the order of *Sac*I DNA fragments as AB–I–D–(G, H)–F–C–E–AB–. The relative positions of *Sac*I fragments G and H were not determined, as none of the restriction enzymes used in this study cleaved these two fragments. An accurate position of the *Sac*I cleavage site between two contiguous fragments of about 20 megadaltons, *Sac*I fragments AB and AB, could not be determined relative to *Eco*RI, as this *Sac*I cleavage site was apparently very close to an *Eco*RI site. The position of this cleavage site was found by reciprocal double digests of E2 DNA with *Sac*I and *Sma*I. *Sma*I-A was cleaved by *Sac*I into *Sac*I fragments AB, C, and E and two end fragments of 4.1 and 0.50 megadaltons (Fig. 5). The 4.1-megadalton fragment was at the right-hand end of *Sma*I-A, thus, the restriction site between *Sac*I fragments AB and AB could be accurately located at 89.9% of the genome (see Fig. 8A).

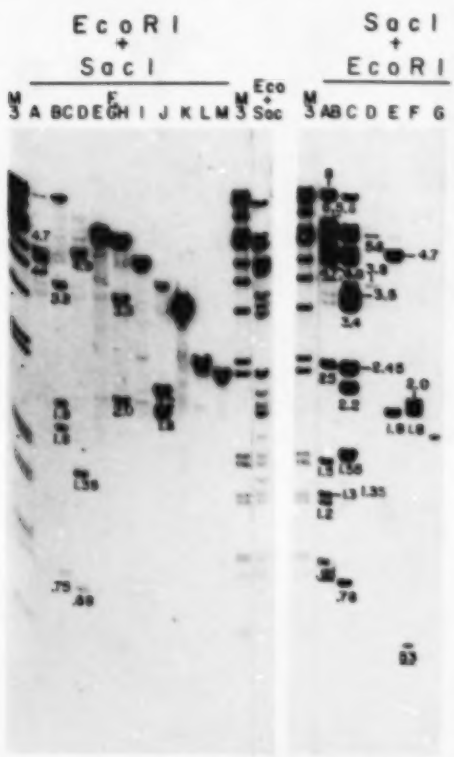

FIG. 6. *Reciprocal digests of AcMNPV M3 EcoRI fragments A through M digested with SacI (EcoRI + SacI) and SacI fragments A through G digested with EcoRI (SacI + EcoRI) were electrophoresed as in previous figures. EcoRI digests of AcMNPV M3 (M3) and digestion of AcMNPV E2 with EcoRI and SacI (Eco + Sac) are shown as molecular weight standards. Fragments are labeled as in Fig. 2.*

***Xho*I cleavage sites.** *Xho*I cleaved Ac*M*NPV E2 DNA into 13 DNA fragments detectable in 0.75% agarose gels. The order of these fragments was determined after analysis of reciprocal double digests of *Xho*I DNA fragments with *Sac*I and *Eco*RI. A partial sequence *Xho*I–B–A–(E, L, M)–H–(F, CD, CD, J, K, I, G) was established by an analysis of reciprocal digests with *Sac*I (Fig. 7). Reciprocal digests of E2 DNA with *Xho*I and *Eco*RI (Fig. 3 and 7) established the relative positions of *Xho*I cleavage sites as shown in Figure 8A. Note that *Xho*I fragments L and M were left unordered, as the *Eco*RI cleavage products of these two fragments were too small to resolve in the gel system used; thus, their relative positions could not be determined. The position of most of the *Xho*I cleavage sites was confirmed by reciprocal digest experiments with *Sma*I (Fig. 5) and *Bam*HI (not shown).

***Kpn*I cleavage sites.** Reciprocal double digests of Ac*M*NPV E2 DNA with *Kpn*I and *Eco*RI (Fig. 3) gave the order of the five *Kpn*I restriction fragments as *Kpn*I-AB–D–E–AB–C. Reciprocal digests of *Kpn*I fragments with *Bam*HI and *Sma*I confirmed this order.

***Eco*RI cleavage sites.** The order of 22 *Eco*RI restriction fragments (see Fig. 8A) was determined after an analysis of reciprocal double digests of Ac*M*NPV E2 DNA with *Eco*RI and the other five restriction enzymes used in this study. These data are presented in Fig. 2 through 7 and have been discussed above. Two *Eco*RI restriction bands which comigrated on analytical agarose gels, *Eco*RI fragments UV and UV, were also contiguous; therefore, the cleavage site between these two bands was midway between 35.4 and 37.2% of the genome, or 36.3% (see Fig. 8A). The order of *Eco*RI fragments O and R, which were not cleaved by the other five restriction enzymes, was established by isolating three partial digest fragments of *Bam*HI-C cleaved with *Eco*RI (Fig. 2) and redigesting each with *Eco*RI. These data (not shown) determined the order as *Eco*RI-A–O–R–I (see Fig. 8A). The

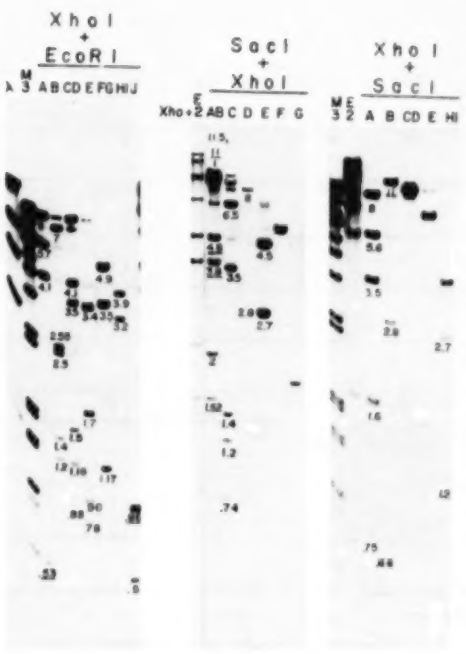

FIG. 7. *Isolated XhoI fragments from AcMNPV E2 were digested with EcoRI (XhoI + EcoRI) and SacI (XhoI + SacI). AcMNPV E2 SacI fragments were digested with XhoI (SacI + XhoI), and each digest was electrophoresed in 0.75% agarose. Molecular weight standards were run in adjacent slots and are labeled as described in Fig. 1. A SacI digest of AcMNPV E2 (E2) is adjacent to the XhoI + SacI reciprocal digest. Fragments are labeled as in Fig. 2.*

smallest *Eco*RI fragment, W, was not detected in any of the reciprocal experiments; however, the following data suggest that it may have been located between *Eco*RI fragments J and A. The cleavage of *Xho*I-H, a fragment of 4.0 megadaltons, with *Eco*RI gave only a single detectable end fragment at 3.2 megadaltons (Fig. 7). The 3.9-megadalton band seen in the same slot in Fig. 7 was uncleaved *Xho*I-I, which could not be separated from *Xho*I-H on preparative agarose gels but which could be resolved on analytical gels (see Fig. 1). If *Eco*RI cleaved *Xho*I-H once, then there should have been, in addition to the 3.2-megadalton band, another end fragment of 0.8 megadaltons. As a DNA band of this size was clearly absent, this suggested that there were at least two *Eco*RI cleavage sites in *Xho*I-H. Therefore, the position of *Eco*RI-W of 0.38 megadaltons could have been within *Xho*I-H and between *Eco*RI fragments J and A.

The cleavage sites of *Eco*RI as well as *Sma*I, *Kpn*I, *Bam*HI, *Sac*I, and *Xho*I that were mapped on Ac*M*NPV E2 DNA are shown in Fig. 8A. To facilitate the comparison of restriction sites, the circular genome of Ac*M*NPV E2 has been illustrated as a linear form in a manner similar to that presented by Subramanian et al. (9) for simian virus 40 DNA. Each restriction site has been labeled as a percentage of the genome with respect to its position and the *Bam*HI-A, 0.0% cleavage site.

Mapping the DNAs of four Ac*M*NPV genetic variants. Reciprocal double digest experiments with Ac*M*NPV S1 DNA were done as described above for E2 DNA. These data (not shown) established that the order as well as the number of Ac*M*NPV S1 *Eco*RI, *Bam*HI, and *Sma*I restriction fragments were the same as those of E2 when compared to the restriction map of E2. Since the Ac*M*NPV S1 *Sac*I, *Xho*I, and *Kpn*I restriction fragment patterns were almost identical to E2 DNA fragment patterns, it was assumed that the order of these fragments was also the same as that already mapped with E2 DNA. The limit digests of S1 [^{32}P]DNA restricted with *Bam*HI, *Kpn*I, and *Sac*I had the same fragment patterns after electrophoresis in 0.75% agarose as described above E2 DNA (Fig. 1). However, the S1 DNA fragments *Xho*I-B, *Eco*RI-D, and *Sma*I-C were about 0.70 megadaltons larger than corresponding E2 fragments. This located an insertion in the S1 genome of 0.70 megadaltons between 14.1 and 16.5% relative to the E2 cleavage map (Fig. 8B). The lower limit of the insertion, 14.7%, was the *Sma*I-C cleavage site, and the upper limit of 16.5% was consistent with the fact that the mobilities of *Sac*I fragments I and D were not affected by the S1 DNA insertion.

A comparison of the limit digests of Ac*M*NPV E2 and M3 with *Xho*I, *Eco*RI and *Sma*I (Fig. 1) and *Sac*I, *Kpn*I, and *Bam*HI (not shown) showed that the only difference in restriction fragment patterns was in the migration of *Eco*RI-L of M3 DNA, which was 0.10 megadaltons larger than the corresponding E2 fragment. The location of this apparent insertion in the M3 variant genome could be deduced from the above data to be between 6.0 and 7.0% relative to the E2 restriction map (Fig. 8B).

The S3 variant of Ac*M*NPV was analyzed in a manner similar to that described above and found to have an insertion of 0.10 megadaltons at 6.0 to 7.0% and another of 0.20 megadaltons at 0.0 to 2.4% when compared with the restriction map of E2 (Fig. 8B).

Comparison of the limit digests of the Ac*M*NPV variant R9 with E2 DNA showed that the R9 genome had an additional *Eco*RI cleavage site in *Eco*RI-A and an additional *Bam*HI cleavage site in *Bam*HI-A (Fig. 1 and 8B).

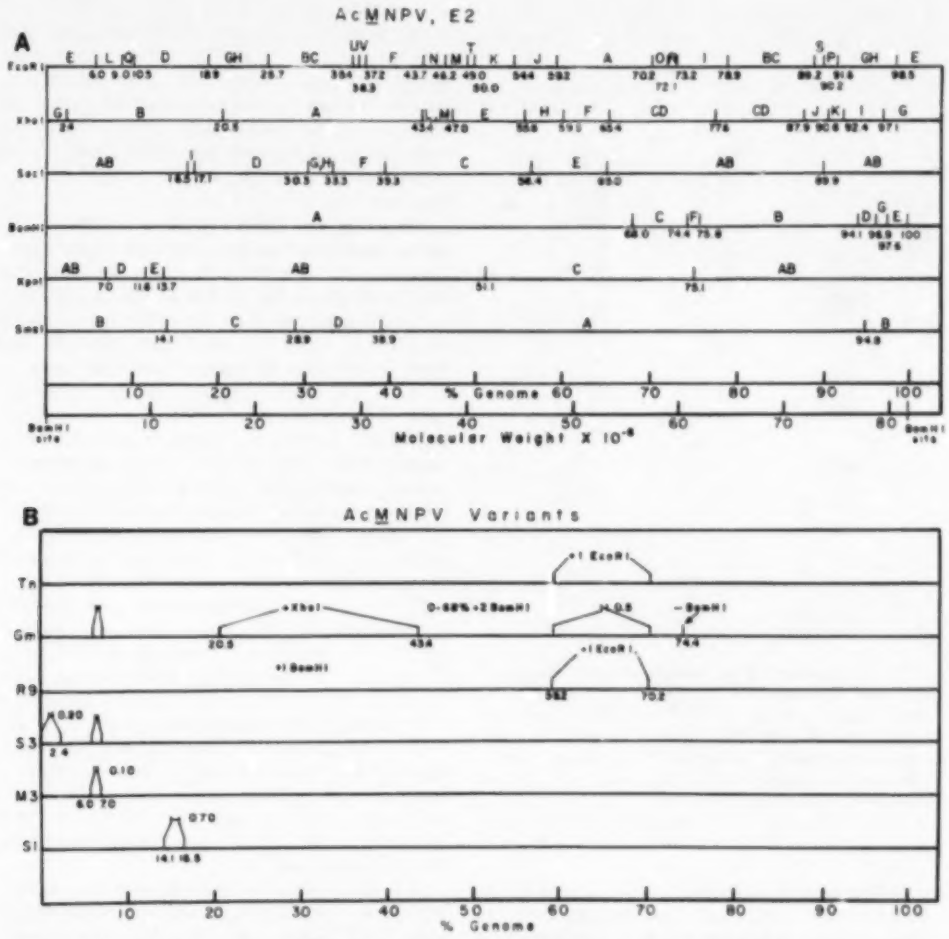

FIG. 8. *(A) Physical maps of AcMNPV E2 DNA derived in this study for the restriction enyzmes SmaI, KpnI, BamHI, SacI, XhoI, and EcoRI. The circular DNA of AcMNPV is presented in a linear form for convenience of comparing the cleavage maps. The horizontal lines refer to the DNA, and the vertical lines indicate cleavage sites. The BamHI-A cleavage site of 0.0% of the genome was used to orient all other cleavage sites. One hundred percent of the genome is equal to a molecular weight of 81.9 × 10⁶. Each cleavage site is labeled as a percentage of the genome and as to its position relative to the 0.0% cleavage site. (B) Physical maps of AcMNPV S1, M3, S3, and R9 variants, GmMNPV, and TnMNPV DNAs relative to AcMNPV E2 DNA. Changes in the number of restriction sites have been indicated as either additional cleavage sites (+) or loss of a particular cleavage site (−). Lines have been drawn to the cleavage sites within which the changes in the numbers of restriction sites have been located. Insertions that have been mapped relative to AcMNPV are labeled as to their molecular weights in megadaltons and indicated as brackets (H) whose lengths have been drawn to scale. Connecting lines show the cleavage sites between which the insertions occur.*

Mapping Tn*M*NPV and Gm*M*NPV DNAs. The restriction digests of Tn*M*NPV DNA when compared by agarose gel electrophoresis to digests of Ac*M*NPV E2 DNA were very similar. No difference was observed in the *Kpn*I, *Bam*HI, *Xho*I, *Sac*I, and *Sma*I restriction patterns of Tn*M*NPV DNA when compared to E2 DNA. The Tn*M*NPV DNA fragment *Eco*RI-A, however, had at least one additional *Eco*RI cleavage site (Fig. 1). The several high-molecular weight minor bands seen in the particular *Eco*RI digest of Tn*M*NPV DNA (Fig. 1) were apparently partial digest products, as they were not observed in subsequent digests.

The restriction patterns of Gm*M*NPV DNA digested with *Kpn*I, *Sac*I, and *Sma*I were the same when compared to those of E2 DNA. However, the Gm*M*NPV genome was apparently missing a *Bam*HI cleavage site between *Bam*HI fragments C and F, as these two fragments were missing and a new fragment of about 6.6 megadaltons was present which was equal to the sum of the molecular weights of *Bam*HI fragments C and F (Fig. 1). Gm*M*NPV DNA also had two additional *Bam*HI cleavage sites in *Bam*HI-A (Fig. 1). The *Xho*I digest of Gm*M*NPV showed that relative to the digest of E2 DNA, the genome of Gm*M*NPV had an additional *Xho*I cleavage site in *Xho*I-A. The *Eco*RI fragments A, F, GH, and L of Gm*M*NPV DNA were also different than the corresponding E2 fragments when electrophoresed in agarose gels (Fig. 1). The *Eco*RI-L fragment apparently had an insertion of 0.10 megadaltons at 6.0 to 7.0% of the genome the same as or similar to those in M3 and S3 DNAs, and *Eco*RI-A in Gm*M*NPV DNA was 0.5 megadaltons larger than that found in E2 DNA (Fig. 8B). The cause of the altered mobilities of *Eco*RI-F and GH was not determined.

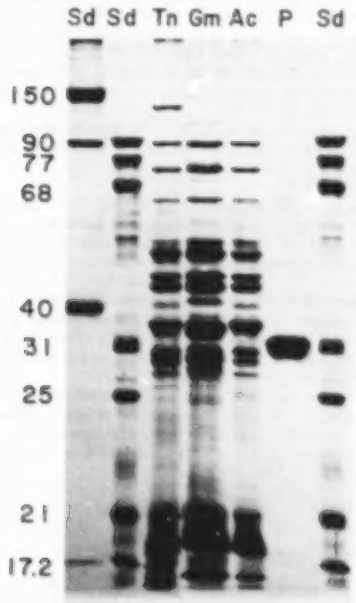

FIG. 9. *Polyacrylamide gel analysis of TnMNPV (Tn), GmMNPV (Gm), and AcMNPV (Ac) structural polypeptides, and AcMNPV polyhedrin (P). About 40 μg of purified virus or 5 μg of polyhedrin protein was disrupted, electrophoresed, and stained as described in the text. Protein molecular weight standards (Sd) electrophoresed in adjacent slots are: RNA nucleotidyltransferase subunits of 150,000, 90,000, and 40,000; phosphorylase, 90,000; lactoperoxidase, 77,000; bovine serum albumin, 68,000; DNase I, 31,000; chymotrypsinogen, 25,000; trypsin inhibitor, 21,000; and myoglobin, 17,200.*

Viral structural proteins. The structural proteins of Ac*M*NPV variants E2, S1, M3, S3, and R9 when compared by polyacrylamide gel electrophoresis were similar to those shown for E2 in Fig. 9. The electrophoretic mobilities of the major structural polypeptides of Gm*M*NPV and Tn*M*NPV were the same as those of Ac*M*NPV E2 (Fig. 9). Tn*M*NPV had a polypeptide of about 17,000 daltons not seen in Ac*M*NPV. Gm*M*NPV had a minor polypeptide of about 28,000 daltons which was apparently absent in Ac*M*NPV, and there was a slight decrease in the mobility of a Gm*M*NPV polypeptide at about 40,000 daltons when compared with Ac*M*NPV. In addition, differences were revealed in several minor polypeptides between 90,000 and 150,000 daltons in Tn*M*NPV and Gm*M*NPV. However, these high-molecular-weight polypeptides may have been aggregates of smaller polypeptides or incompletely reduced proteins (10). The polyhedrin proteins of Ac*M*NPV variants, Tn*M*NPV, and Gm*M*NPV all had the same apparent molecular weight of about 30,000 after electrophoresis in polyacrylamide gels.

DISCUSSION

The E2 variant of Ac*M*NPV was chosen for restriction endonuclease mapping because the E2 DNA restriction bands of the six restriction endonucleases used in this study were most like those of the wild-type Ac*M*NPV DNA. In fact, of 41 plaque-purified isolates from this laboratory (8), 32 had the *Eco*RI restriction pattern of E2 DNA. The DNA of 5 plaque-purified virus isolates had the *Eco*RI restriction pattern of M3. The *Eco*RI cleavage patterns of S1, S3, and R9 DNAs were each found once in the analysis of the same group of 41 plaque-purified isolates. Selection of the E2 genome as the parent type was arbitrary and done to allow a convenient means of comparing the cleavage maps of the other closely related genomes, not to imply that the evolutionary relatedness of Ac*M*NPV variants is known. As yet, no biological or structural protein change can be correlated to the differences in the genomes of the five Ac*M*NPV variants, Tn*M*NPV, and Gm*M*NPV. One interesting feature is a 0.10-megadalton insertion at 6.0 to 7.0% of the genome found in Ac*M*NPV variants M3 and S3 and Gm*M*NPV DNAs. Although only capable of coding for about 50 amino acids, it may prove to be of functional significance, as it is present in about 10 to 20% of the wild-type

AcMNPV genomes (8) and is also found in the genome of GmMNPV, which was acquired from a source other than our laboratory.

The close relatedness of the genomes and structural polypeptides of AcMNPV variants to GmMNPV and TnMNPV suggests that those criteria for identification that have been historically used to classify baculoviruses need to be reevaluated. A similar conclusion is provided by Miller and Dawes (5), comparing the restriction digests of AcMNPV and TnMNPV. For example, the TnMNPV used in that study was not identical to the one compared in this report.

Restriction enzyme maps will prove to be of great value when used to analyze the structure and function of baculovirus genomes. In particular, cleavage maps will be applied in the derivation of transcriptional and translational maps, mapping control sites, such as the origin(s) of DNA replication, mapping of mutation and recombination sites, and ultimately in mapping those biological functions involved in host range specificity.

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ADDENDUM IN PROOF

A recent publication by Miller and Dawes (J. Virol. **29**:1044–1055, 1979) determined the order of *Bam*HI and *Xma*I (an isoschizomer of *Sma*I) restriction fragments and partially ordered the *Eco*RI fragments of a genetic variant of AcMNPV, L1. When these data are compared with the restriction maps of AcMNPV E2, the order of *Bam*HI and *Sma*I restriction fragments is the same. The partial order given for AcMNPV L1 *Eco*RI restriction fragments agrees with the more complete restriction map of AcMNPV E2, with the exception of our *Eco*RI fragment V; thus, the E2 variant of AcMNPV mapped in this laboratory is very similar to the L1 variant reported by Miller and Dawes.

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